NERVOUS CONTROL OF THE LUNGS:

BRONCHO - AND VASO MOTOR RESPONSES OF GUINEA-PIG LUNGS, PRODUCED BY DRUG INJECTIONS AND NERVE STIMULATIONS.

by

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CONTENTS.

I.P.P. = Intrapulmonary pressure.
V.O. = venous outflow.

BRONCHO- AND VASO-MOTOR RESPONSES OF GUINEA-PIG LUNGS.

INTRODUCTION.

It has been established that the pulmonary vaso-motor and broncho-motor nerves are functionally active, but it is not at all clear how far broncho-motor activity may exert a passive effect on the blood vessels by direct compression or by broncho-constriction leading to retention of air in the alveoli and thus causing compression of the alveolar capillaries.

The literature dealing with pulmonary vaso-motor activity has been reviewed on more than one occasion: (Wiggers 1931; Nero 1930; Daly 1932; Daly and Euler 1932) and it has been shown by the last named authors that stimulation of the pulmonary nerves in the dog leads on occasions to gross changes in the pulmonary arterial pressure without alterations in the magnitude of tidal air. They concluded from these experiments, that vaso-motor changes can take place without intervention of broncho-motor effects, but that the independence of vaso-motor and broncho-motor mechanisms is not excluded.

Working on the guinea-pig Dale and Narayana(1935) found that adrenaline had a direct constrictor action on the blood vessels, and also a broncho dilator effect, which caused a passive vaso dilatation. They concluded that/

that the observed effects of adrenaline action on the pulmonary flow were determined by the algebraic sum of these two factors.

This section of the thesis describes and attempts to interpret the results of experiments on the lungs of the guinea-pig designed to determine how far broncho- and vasomotor effects are dependent upon one another, and to test whether unequivocal evidence can be obtained of the independence under certain conditions of experimentation. The methods adopted for attacking this problem involved the injection of drugs and the stimulation of the cervical vagus and cervical sympathetic nerves in an attempt to separate from one another the broncho- and vasomotor responses.

The experiments were carried out in Edinburgh between February and June on mostly male guinea-pigs from a white stock inbred for 4 years. These animals each weighed between 600 - 750 grams. Their normal diet consisted of hay, cabbage and special meal mixture - oats, bran, ground wheat, ground barley, soya bean meal, and linseed cake meal. Beetroot was given once a week in February and March. The method of perfusion of the isolated lungs was similar to that described by Daly, Peat and Schild (1935). The animals were killed by a blow on the neck. Cannulae were inserted into the trachea, pulmonary artery and the left auricle. In nerve stimulation experiments the cervical-vago-sympathetic (C.V.S.) nerves were exposed, and when necessary separated/

FIG. 1.

A side arrangement to convert a constant flow perfusion of lungs into one of constant pressure.
P.A. = pulmonary arterial tubing leading from a constant flow pump to the pulmonary artery of the animal. From it a T-piece is connected to the constant pressure reservoir C.P. which is a T-shaped tube 1 cm. in diameter, 5 cm. in height and open at the atmosphere. From this an overflow spout (O) directs the drops over a pair of electrodes (2) into the venous reservoir V.R. which can be fixed at any desired height by means of a sliding metal clip (S.A.) on to a wooden attachment on the inside of the tank.

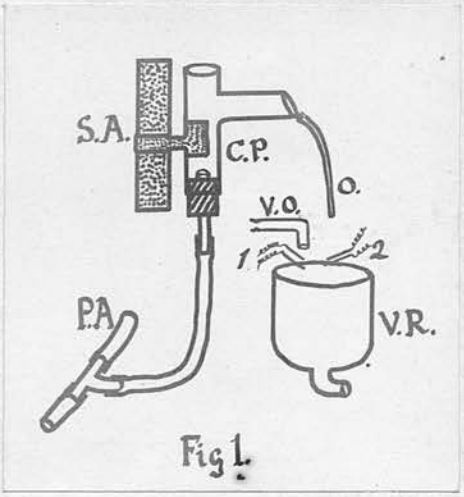

Fig 1.

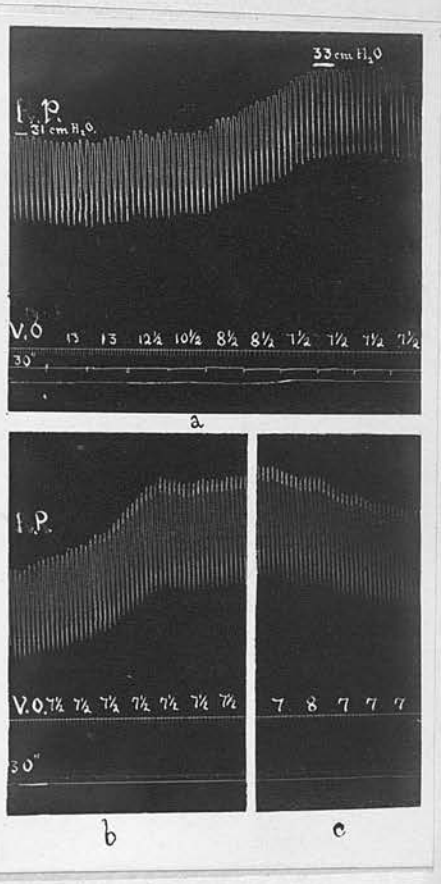

Fig.2

FIG. 2. 25.2.37.

Spontaneous variations which frequently occur in Isolated Perfused Lung Preparations of a guinea-pig.

a. shows a rise in Intrapulmonary Pressure accompanied by a fall in venous outflow.
b. shows a tracing from a different animal; a rise in Intrapulmonary pressure unaccompanied by any changes in venous outflow.
c. same animal as in b.; a fall in respiratory pressure unaccompanied by any change in venous outflow.

I.P.P. intrapulmonary pressure. ("respiratory" pressure).
V.O. venous outflow from the left auricle.
The figures of the V.O. tracing indicate drops per ½ minute.

separated into the component vagal and sympathetic divisions immediately prior to placing the animal in the respiration tank, which was kept at a constant temperature of 34-37°C. by means of a thermostat.

The perfusion and artificial respiration of the lungs was carried out with the micro-perfusion blood and air pumps described by Daly (1937). The lungs were perfused with approximately 20 cc. of Hypertonic Tyrode solution of the following composition: NaCl 0.8 g., KCl 0.02 g.; Na HCO_3 0.1 g. per 80 cc. distilled water for all experiments except Numbers 16-19. The p$_H$ of the perfusing fluid was 8.4 to 7.2 or 8.2 to 7.2. In Experiments 16-19 was used an isotonic solution of the above composition but in 100 c.c. of distilled water. The fluid was kept in the venous reservoir of about 8 c.c. capacity; which was also in the tank and therefore at constant temperature. From this the fluid was propelled onwards by means of 3 pistons compressing the tube at such time intervals as to simulate the action of the heart. The rate of the pump could be raised at will but once set it remained fixed for the given series of experiments. Inside the tank a vertical tube led off the pulmonary arterial tubing, and thus provided a means of establishing a constant perfusion pressure. (Fig. 1.) The side tube was provided with a spout to direct any overflow into the venous reservoir. The perfusion pressure varied

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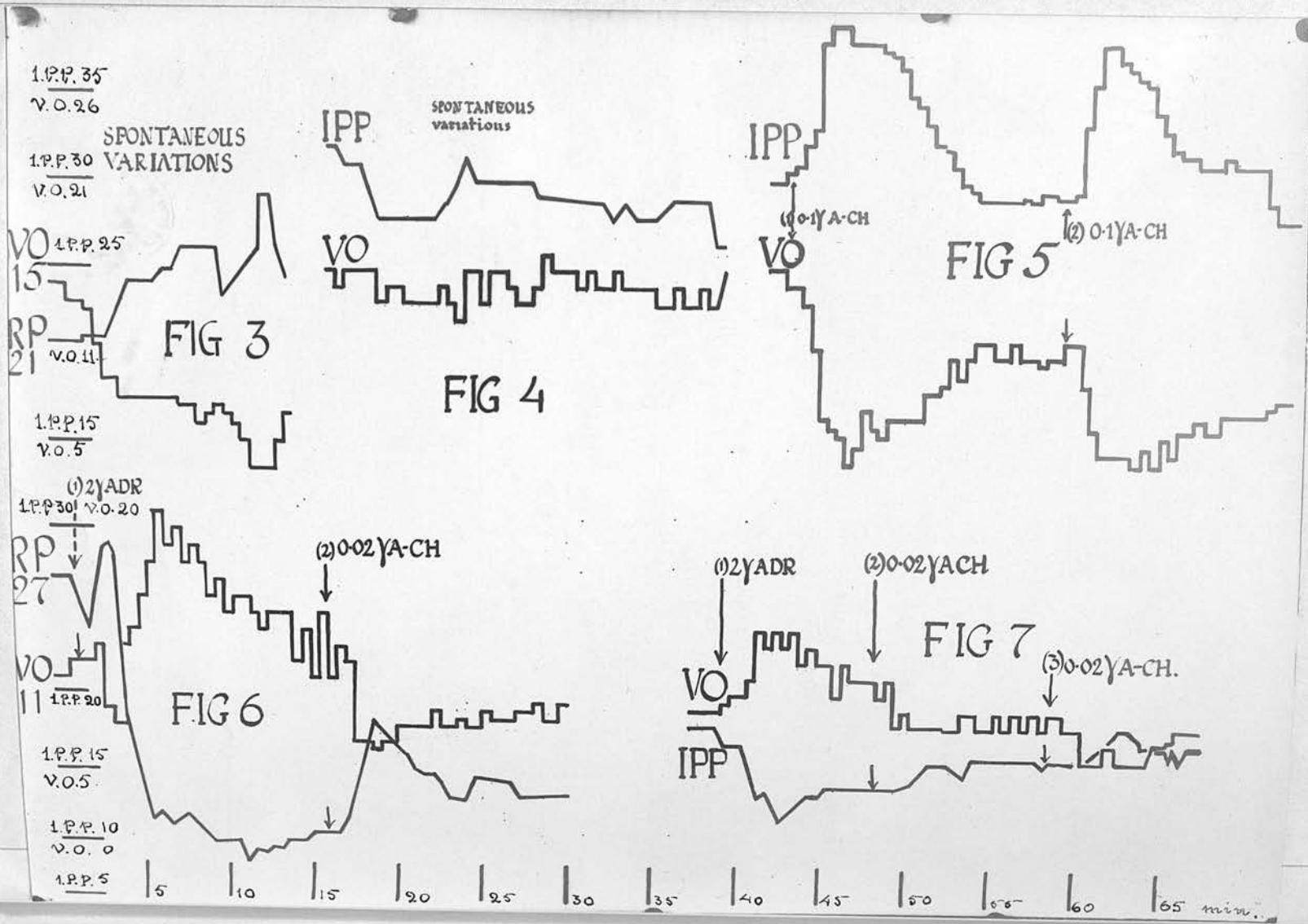

1.P.P. 35
V.O.26
SPONTANEOUS VARIATIONS
1.P.P.30
V.O.21
VO
1.P.P.25
15
RP
21
V.O.11
1.P.P.15
V.O.5
FIG 3
IPP
SPONTANEOUS variations
VO
FIG 4
IPP
(1) 0·1γ A-CH
VO
(2) 0·1γ A-CH
FIG 5
(1) 2γ ADR
1.P.P.30
V.O.20
RP
27
VO
11
1.P.P.20
(2) 0·02γ A-CH
FIG 6
1.P.P.15
V.O.5
1.P.P.10
V.O. 0
1.P.P.5
5
10
15
20
25
30
35
40
45
50
55
60
65 min.
(1) 2γ ADR
(2) 0·02γ ACH
FIG 7
(3) 0·02γ A-CH.
VO
IPP

FIG. 3. 25.3.37. I.P.L. (Isolated Perfused Lungs) of guinea-pig 15; showing spontaneous variations in Intrapulmonary Pressure and the venous outflow, which has been frequently known to occur in our preparations. The numerals referring to the I.P.P. and V.O. indicate cm. H_2O and drops per 30 sec. respectively.

FIG. 4. 17.3.37. I.P.L. of guinea-pig 11. Spontaneous variations in Intrapulmonary Pressure and the venous outflow.

FIG. 5. 25.2.37. I.P.L. of guinea-pig 7. At (1) 0.1 γ ACH injected into the P.A. In response to each injection the I.P.P. rises and the V.O. falls in an almost symmetrical and inverse ratio to each other. Both the I.P.P. and V.O. return after a time to approximately the initial levels. It also shows that an injection of ACH does not materially affect the value of any response to subsequent doses of ACH.

FIG. 6. 11.3.37. I.P.L. of guinea-pig 10. At (1) 2 γ Adr. was injected into the P.A. and produced a fall in I.P.P. after a preliminary small fall. The I.P.P. fall is associated with an increase in venous outflow. At (2) 0.02 γ ACH injected into P.A. causes a rise in I.P.P. and a fall in venous outflow.

FIG. 7. 11.3.37. I.P.L. of guinea-pig 10. At (1) 2 γ Adrenaline injected into the P.A. produces a fall in I.P.P. and an increase in V.O. At (2) 0.02 γ ACH was injected into the V.R. At (3) 0.02 γ ACH injected per P.A. produce changes which are too small to interpret.

RESULTS.

I. Spontaneous variations in I.P.P. and V.O. (venous outflow.)

In a few experiments it was noticed that the I.P.P. rose spontaneously with or without accompanying changes in the V.O. (Figs. 2, 3 and 4.) We will discuss the significance of this observation later on in relation to the question of the independence of bronchomotor and vasomotor mechanisms. Here it is sufficient to record that the I.P.P. may rise or fall spontaneously without producing an alteration in V.O. or it may rise spontaneously with a diminution in V.O.

II. Acetyl Choline (A.Ch.)

Dale and Narayna (1935) working on isolated perfused lungs (I.P.L.) of the guinea-pig in which the perfusate was not recirculated, found that A.Ch. produced a rise in I.P.P. generally associated with a reduction in venous outflow. Since A.Ch. (and vagal stimulation) produced vaso-constriction when the lungs were permanently inflated by a positive intrapulmonary pressure, they concluded that the action of A.Ch. on the outflow could take place independently of the bronchomotor mechanisms although it could be affected by an increase in the intrapulmonary due to bronchoconstriction causing a retention of air in the alveoli and passive compression of the blood vessels.

We/

TABLE I.

INJECTIONS of ACETYL - CHOLINE.

G.P. NO.	PERF. P.	DOSE	INITIAL I.P.P.	LATENT PERIOD	EFFECT ON I.P.P.	INITIAL OUTFLOOD	LATENT PERIOD	EFFECT ON OUTFLOOD.	INITIAL I.P.P. RANGE.
						8 drops /30"			
10	2cm.	(5)0.02 γ	6.5 c.m.	1 min.	+		within 30"	-	0 - 10 cmH20
10	2	(2)0.02 γ	4.5	1 m.	+	14	1 m.	-	10-20 cmH20
12	5	(4)0.01 γ	12	w.30"	+R.I.L.	12	1.25 m.	-RIL	
10	2	(2)0.02 γ	13.5	1 m.	+ RIL	10	1 m.	-RIL	
9	5	(5)0.02 γ	16	1.5m	+ RIL	9	1.5 m.	-RIL	
		(2)0.02 γ	17	1 m.	S- + RIL	11	1 m.	-RIL	
							?	?	
13	4	(4)0.02 γ	18	1 m.	S+	11			
		(11)0.02 γ	19.5	1.5m.	+	8.5	w.30"	- ?	
12	7	(10)0.02 γ	20	-	+	6.5	-	- ?	20 -30 cmH20.
9	7	(1) 0.02γ	21	1 m.	+	17	30"	-	
6	8	(8)0.1 γ	21	1 m.	+	12	w.30"	-	
8	5	(3)0.03γ	21.5	1 m.	+ RIL	9.5	1.5 m.	S-RIL	
		(5)0.3 γ	21.5	1 m.	+ RIL	10	1.75 m.	- RIL	
6	8	(11)0.1 γ	22.5	1 m.	+ RIL	10	1.75 m.	- RIL	
11	3	(1)0.002γ	26	1 m.	+	14	45"	-	
7	2	(2)0.1 γ	29.5	w.30"	+ RIL	11	w.30"	-	
		(4)0.1 γ	29.5	1 m.	+ RIL	6	1 m.	-	
		(1)0.1 γ	30 cm	w.30"	+ RIL	15	w.30"	- RIL	
		(3)0.1 γ	32 cm	1 m.	+ RIL	7.5	30"	- RIL	
14	4.5	(2)0.0001γ	31 cm.	3 cm.	-	21	2.5 m.	+ RIL	
17	7.5	(5)0.01γ	31 cm.	1 cm.	+	18	1.25m.	+	

G.-P. NO = Number of Guinea-Pig as in records. Perf.P. = Perfusion Pressure. Initial I.P.P. = Initial Intra Pulmonary Pressure. W. = within the period of

All injections made into the Pulmonary artery except where stated into the venous reservoir.

R.I.L. = return to the initial level.

We performed altogether 33 injections of A.Ch. (Hoffman- LaRoche and Co.) into the pulmonary artery, each injection being never more than 0.2 c.c. in volume. 21 responses were obtained and their detailed nature is summarised in Table I.

An examination of these results reveals that A.Ch. appears consistently to raise the I.P.P. which is almost always associated with a reduction in the venous outflow (V.O.) namely:

I.P.P. +	V.O. -	17	tests.
I.P.P. +	V.O? -	3	"
I.P.P. -	V.O. +	1	"

If each experiment is plotted out on graph paper, it appears that the variations in I.P.P. and V.O. are almost invariably inversely related to each other. (Figs. 5, 6, 7 and 8). Moreover, as the experiment progresses a complete return to the initial level takes place both with the I.P.P. and V.O. But occasionally the raised I.P.P. level remains raised and the V.O. also stays diminished.

The typical "I.P.P.+ V.O. -" response seems to take place independently of the initial level of the I.P.P.; of the size of the A.Ch dose administered (range 0.002 γ to 0.3 γ); or of the initial perfusion pressure value. (Fig. 9) The latent periods of the responses are approximately the same for the I.P.P. and V.O. The majority range from 30" to 1.5 min.; in 2

cases/

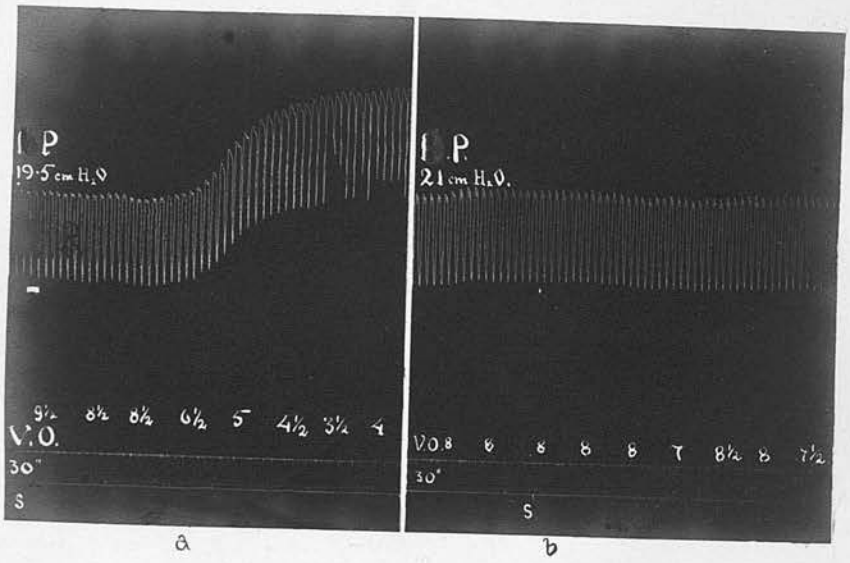


Fig 10

FIG. 10. 19.3.37. Guinea-pig No. 13.

To show the effect of injected Acetyl Choline before and after Atropine in an Isolated Lungs preparation of a guinea-pig.

a. At signal S, 0.002 γ Acetyl Choline Chloride was injected into the Pulmonary Arterial Tubing. Between a. and b. 300 γ Atropine Sulphate injected into the venous reservoir.

b. At signal S, 15 min. after Atropine injection 0.002 γ Acetyl Chlorine Chloride injected into the Pulmonary Arterial tubing. The effect of Acetyl Choline has been abolished by Atropine.

I.P. = intrapulmonary pressure.
V.O. = venous outflow from the left auricle.

TABLE II.

NUMBER OF GUINEA PIG and P.p.	INJECTIONS: DOSES AND TIMES	EFFECT ON I.P.P	NET RESULT	EFFECT ON OUTFLOW	NET RESULT
No. 7 P.A.p 2 cm.	(1) o.1 γ ACH/P.A. at 2.40 p.m.	30cm → 39 cm	+9 cm	15 drops/30" →4	- 11
	(2) 0.1 γ " " " 2.55 p.m.	29.25 → 36.5 cm	+8.75cm	11 →4	- 7
	(3) 0.1 γ " " " 3.30 p.m.	32 → 36 cm	+4 cm.	6.4 →3	- 3.4
	(4) 0.1 γ " " " 3.45 p.m.	29 → 36 cm	+7 cm.	6 →4	- 2
No. 8 P.A.p. 5 cm.	(3) 0.03 γ ACH/P.A. at 3.30 p.m.	21.6 → 26 cm.	+5.5 cm	10 →8	- 2
	(4) 2 γ ADRENALINE/P.A at 3.45 p.m.	21.5 → 22.8 cm	+1.3 cm	10 →8	- 2
	(5) 0.03 γ ACH/P.A. " 4 p.m.	21.5 → 26 cm	+4.5 cm	10 →8	- 2
No. 9 P.A.p. 2 cm.	(2) 0.02 γ ACH/P.A. at 10.55 a.m.	16.25 → 21,9cm.	+4.65 cm	11 →5	- 6
	(3) 2 γ ADRENALINE/P.A." 11.5 a.m.	18 → 11.7 cm	-6.3 cm	8 →14	+ 6
	(4) 0.02 γ ACH " 11.20 a.m.	14 → 16 cm	+2 cm	11 → 8	- 3
No. 10 P.A.p. 2 cm.	(2) 0.02 γ ACH/P.A. at 10.25 am.m.	2.75 → 9.6cm.	+6.85 cm	14 →6.5	- 7.5
	(3) 0.02 γ ACH/P.A. " 10.45 a.m.	13.5 → 22.4 cm	+ 8.9 cm	11 →6.5	- 4.5
	(4) 1 ADRENALINE/P.A. "11 a.m.	13.9 → 16 cm	+ 2.2 cm	11 →7.5	- 3.5
	(5) 0.02 ACH/P.A. "11.15 a.m.	14.6 →30 cm	+15.4 cm	9.5 → 2	- 7.5

cases it is 1.75 min., and in only one case 3 min. for I.P.P.and 2.5 for V.O.

Eserine was found to have no enhancing action; no doubt due to the blood esterase being all removed in the preliminary washing out of the vessels during preparation. The action of Atropine on A.Ch. effects was found in Exp. 13 to abolish both the I.P.P. rise and the diminution of flow through the vessels (Fig. 10.)

A side issue was raised as to whether the I.P.P. and V.O. effects were potentiated (A) by previous injections of the drug itself; (B) by adrenaline. Dale and Narayana found A.Ch. flow diminution to be potentiated by the addition of Adrenaline to the perfusate. They recalled the findings of Dale and Gaddum (1930) that adrenaline enhances the A.Ch. action in denervated muscle by direct sensitisation. (Isolated strips of the denervated diaphragm of the cat were used). Table II analyses 4 experiments designed specially for this purpose. It will be seen that an injection of A.Ch. does not materially affect the value of any response to subsequent doses of A.Ch. (Fig.5), and that the adrenaline potentiation of A.Ch. effects does not occur when single small doses of adrenaline are used. Fig. II illustrates the changes occurring in guinea-pig No.8 as a result of placing an injection of adrenaline 2.0 γ between injections of A.Ch. of 0.03 γ .

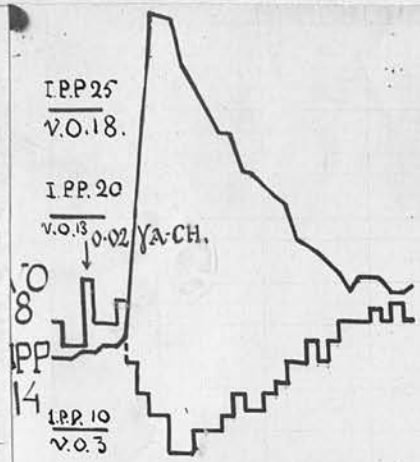


FIG 8

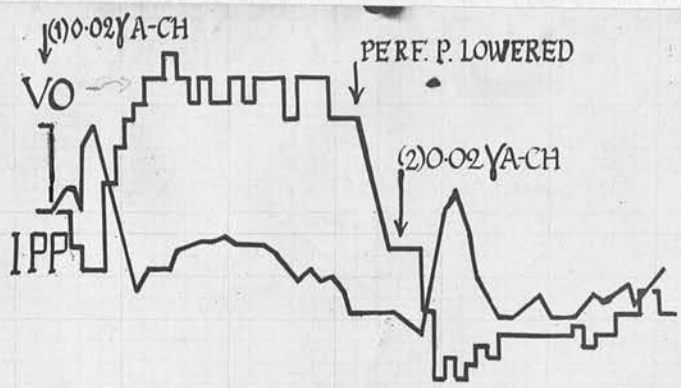


FIG 9

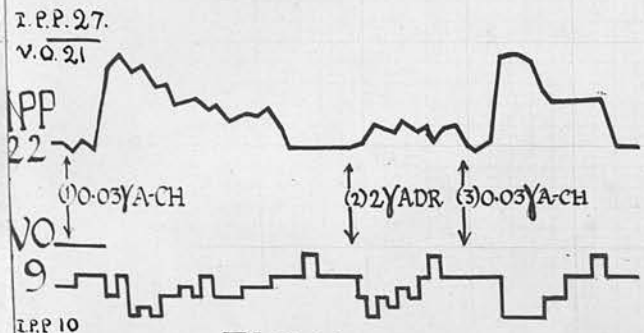


FIG 11

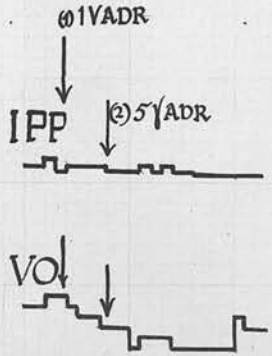


FIG 12

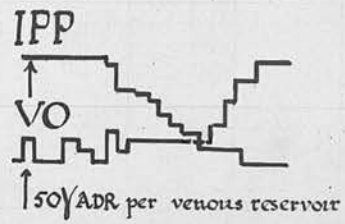


FIG 13

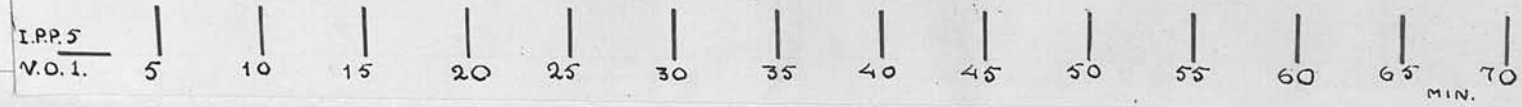


FIG. 8. I.P.L. of a guinea-pig. 0.02 γ ACH. was injected into the pulmonrary artery and produced the typical response - a rise in I.P.P. and a decrease in V.O.

FIG. 9. 10.3.37. I.P. Lungs of guinea-pig 9. At (1) 0.02 γ ACH. with P.A. produced a rise in I.P.P. and a decrease in V.O. At (2) 0.02 γ ACH. were injected into P.A. and produced a similar result. Just prior to the second injection perfusion pressure was lowered from 7 to 5 cm. Therefore the "typical L.P.P.+ VO - " response to ACH. is within the above limits at least independent of the initial perfusion pressure value.

FIG. 11. 9.3.37. I.P.L. of guinea-pig 8. (1) 0.03γ ACH injected into P.A. (2) 2 γ ADH into P.A. (3) 0.03 ACH. injected into P.A. The curves show firstly that the injection of adrenaline causes a rise in I.P.P. associated with a fall in V.O.; and secondly that interposition of an adrenaline injection between two injections of ACH. does not potentiate the second ACH. response.

FIG. 12. 19.2.37. I.P.L. of guinea-pig 6. 1 γ and 5 γ Adrenaline / P.A. at (1) and (2) respectively, together produce a diminution in V.O. with no change in the I.P.P. This is an example of a dissociation of vascular and bronchial responses in perfused lungs.

FIG. 13. 19.2.37. I.P.L. of guinea-pig 6. At arrow 50 γ Adr. were injected into the venous reservoir. The I.P.P. falls and the V.O. increases after a latent period of 5 min. subsequent to the injection. The curves suggest that the two changes are unassociated and that the increase in venous outflow may have been a spontaneous effect; in which case this is a further example of "a dissociation" in which adrenaline may produce a fall in I.P.P. without an immediate change in V.O.

III. Adrenaline.

The results with Adrenaline have been somewhat uncertain. Previously Dale and Narayana (1935) studied both the I.P.P. and V.O. effects of Adrenaline injections. They concluded that Adrenaline always produced a fall in I.P.P. (unless the bronchi were fully dilated.) with V.O. effects which depended "to some extent on the state of tone of the bronchial tubes at the time of the injection. If the I.P.P. was low, injections of Adrenaline always produced a decrease in outflow, which was not accompanied by any significant change in I.P.P. On the other hand if the I.P.P. was high, thus indicating broncho-constriction injection of Adrenaline over the same range of doses always produced a fall in I.P.P. due to broncho-dilatation which was occasionally accompanied by an increase in the outflow. They concluded and later supported their conclusion by additional experiments that an Adrenaline increase of outflow is due to mechanical causes. They could not confirm the existance of seasonal variations in Adrenaline response described by Ettinger (1929, 1931) nor a difference in the action of pure adrenaline and adrenaline with Chloretone.

Unfortunately with the exceptions mentioned above, previous workers have not investigated the I.P.P. and V.O. responses simultaneously. Baehr and Pick (1913) observed/

TABLE III.
INJECTIONS OF ADRENALINE.

GUINA PIG NUMBER	PERFUSION PRESSURE	DOSE OF ADRENALINE	INITIAL I.P.P.	EFFECT ON I.P.P.	EFFECT ON OUTFLOW.
1o	2 cm.	(4) 1 γ /P.A.	13.5 cm.	+	-
9	5	(3) 2 γ /P.A.	18	-	+
6	8	(7) 10 γ / P.A.	20	0	-
		(3) 50 γ /V.R.	24	-	Very late +
		(2) 15 γ /V.R.	25	S+: -	+
		(7) 10 γ /P.A.	19	0	-
		(9 & 10) 1 γ then 5 γ	22.5	o	-
8	5	(4) 2 γ /P.A.	21.5	S +	Def. -
10	10	(1) 2 γ /P.A.	27	S - + def -	S + - big +
19	5	(11) 5 γ /P.A.	30	Sl +	0
		300 γ Ergotoxine			
		(13) 5 γ /P.A.	23	-	0 ?
		(11) 2.5 γ /P.A.	20	V.Sl. +	Sl -

I.P.P. = Intrapulmunary pressure. P.A. = Pulmonary artery. V.R. = Venous Reservoir.
S. Sl.= Slight v. sl. = Very slight.

i.e.	I.P.P. 0	V.O. -	in 3	cases.
	I.P.P. +	V.O. -	3	
	I.P.P. +	V.O. 0	1	
	I.P.P. + -	V.O. +	1	
	I.P.P. -	V.O. + *	2	
	I.P.P. -+-	V.O. +-+	1	

* One of these a late event.

observed no effects of Adrenaline on guinea-pig lungs performed with Tyrode solution. Tribe (1912) obtained vaso-constriction with pure adrenaline and dilatation with Adrenaline containing Chloretone. Ettinger (1929) (1931) observed either a strong constriction or a weak dilatation, depending on seasons of the year.

In the present series of experiments 25 injections of Parke Davis and Co. Adrenaline diluted in Warm Tyrode Solution were made and 25 responses obtained. The drug was injected per pulmonary artery in concentrations of 1 γ per 0.1 cc. Only on two occasions was the drug injected into the venous reservoir (V.R.). The doses varied from 1 γ - 5 γ /P.A. and 15 γ - 50 γ / V.R. The initial I.P.P. varied from 13.5 - 31 cm.H_2O. The results are summarised in Table III, from which it will be seen that adrenaline produced the remarkable effect of broncho-constriction in at least four experiments. Apart from this unexpected result, there appear other significant adrenaline reactions which are illustrated by Figs. 6, 7, 11, 12, 13, 14, and may best be summarised as follows:-

(1) An absence of I.P.P. response with a diminution in V.O. (Fig. 12)

(2) A fall in I.P.P. accompanying a rise in V.O. (Figs. 6, 7).

(3) A slight rise in I.P.P. accompanying a slight fall in V.O. (Fig. 11).

(4)/

(4) A fall in I.P.P. without any concomitant change in V.O., but an augmentation of V.O. occurs five minutes after the commencement of the I.P.P. rise (Fig. 13 and 14). This may have been a spontaneous effect.

Observations (1) and (2) confirm the results of Dale and Narayana (1935) but those subsequently described are new and we will proceed to examine them further.

In the I.P.L. of the guinea-pig the method employed in the past by all workers as far as we can ascertain, involved the continuous replacement in the Lungs of Ringer-Locke solution by fresh solution. It is to be expected therefore that the lungs will continually lose tissue substances to the perforate. Under these conditions adrenaline, if effective, always produces broncho-dilatation. With the method employed in our investigation, a closed circuit perfusion apparatus is used and the perfusate circulates with the result that the loss of tissue substances from the lungs is minimised. The conditions produced by a continuous replacement of fluid as distinct from those induced by a recirculation of the perfusate may have a profound influence on the responses of the lungs, and it is suggested that the observed adrenaline broncho-constriction may be in some way concerned with the retention/

retention of tissue substances in the perfusate. In view of the fact that spontaneous broncho-motor effects are frequently observed when the closed circuit method are used, and only rarely seen with replacement perfusion, again suggests that the appearance of this phenomenon also may be dependent upon the retention of lung tissue substances in the perfusate. In this connection broncho-constriction response to adrenaline in the dog has been repeatedly observed in other so far unpublished experiments in our laboratory, but it cannot be said that the conditions which govern this type of response in the dog or guinea-pig are at all clear. Whatever the ultimate solution may be it seems that the phenomenon in the guinea-pig has thrown very little light upon the problem of independence of bronchomotor and vasomotor actions, one of the problems which we set out to investigate.

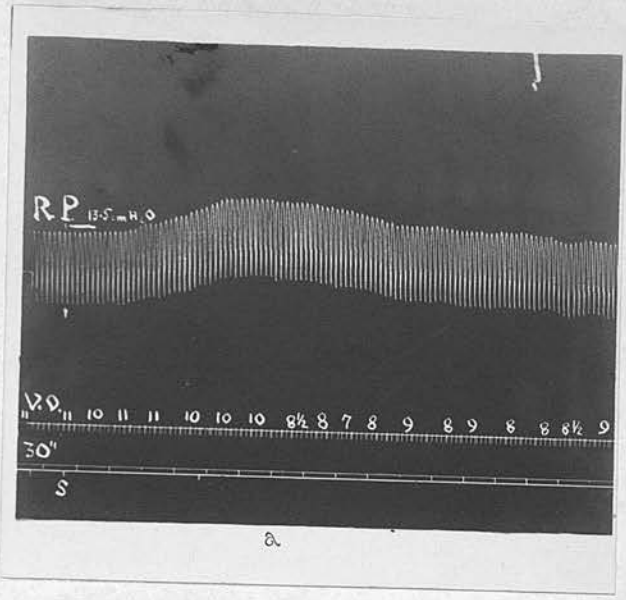
All the results in table III can be accounted for by adrenaline bronchodilatation with which is associated vasoconstriction or vasodilatation, depending upon whether the active vasoconstriction or passive vasodilatation (due to bronchoconstriction) predominates.

Bearing in mind that there can be no certainty of spontaneous variations in I.P.P. and V.O. being absent during the adrenaline responses, our results must be interpreted with caution. Even so, no evidence is forthcoming from the investigation which would lead us to/

to believe that the earlier conclusions of Dale and Narayana (1935) require modification. We confirm them therefore in that an adrenaline broncho dilatation may passively reduce the resistance of the pulmonary vascular bed and so increase the venous outflow, an action which may be masked or reversed by the direct vasoconstriction action of adrenaline which reduces the venous outflow.

The fact that adrenaline may produce a fall in I.P.P. without an immediate change in the V.O. is (Fig. 13) difficult to account for unless it is assumed that the territory of the vascular bed which is usually passively affected by bronchomotor effects is already fully dilated, but the later vaso dilatation observed is due to the response of a totally different portion of vascular Territory.

Before leaving the discussion on the results of adrenaline injections, one other point demands attention. With regard to the potentiation of A.Ch. vaso constriction by perfusates containing 1/250,000 adrenaline, reported by Dale and Narayana (1935), it is found that single injections 1-2 γ Adrenaline interposed between the A.Ch. effects is ineffective in producing potentiation - a point not without interest since it has been suspected in another connection that the imposition of a background on a tissue by a steady infusion of a dilute solution of a drug, may produce a state in the tissue which differs from that produced by a series of single injections of that drug.



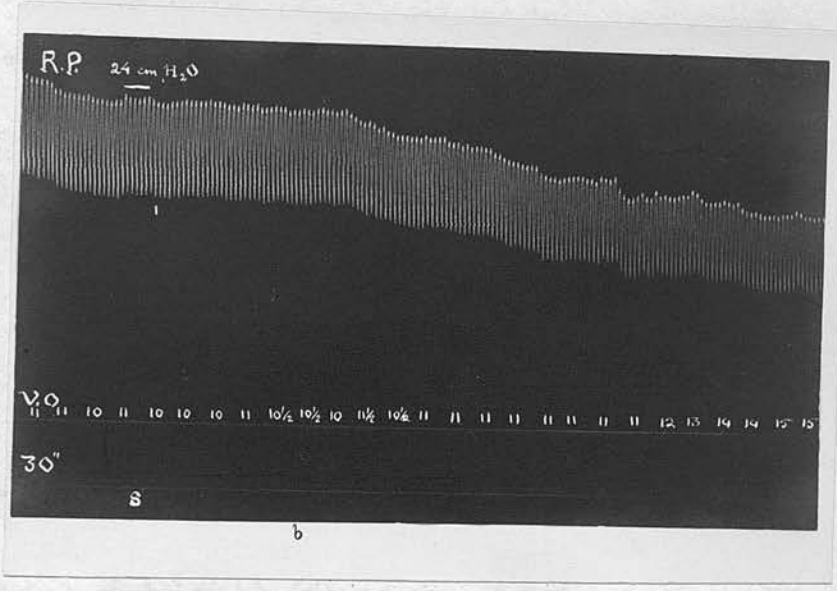


Fig 14 a and b.

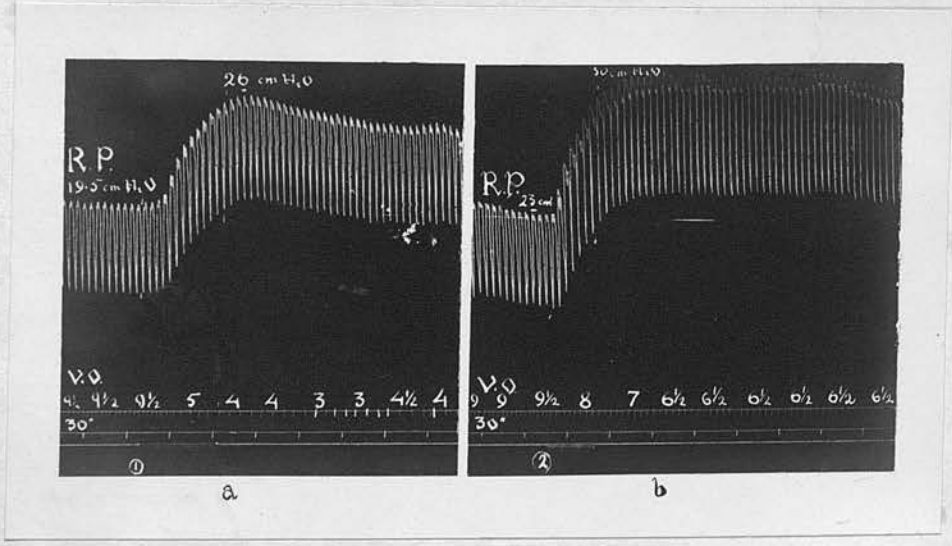


Fig 15.

FIG. 14. Isolated Pertused Lungs Preparation.

a. 11.7.37. Guinea-pig 10. At signal S, 1 γ Adrenaline Chloride injected into the pulmonary arterial tubing. Intrapulmonary pressure rises slightly, the venous outflow falls slightly.

b. 19.2.37. Guinea-pig 6. At signal S, 50 γ Adrenaline Chloride injected into the venous reservoir. Intrapulmonary pressure falls: the venous outflow shows at first no change, then after 9 min. an increase takes place.

R.P. = intrapulmonary pressure.
V.O. = venous outflow from left auricle.

FIG. 15. 25.3.37. Guinea-pig 15. Isolated Perfused Lung preparation.

a. at signal S, peripheral ends of both cervical vago-sympathetics stimulated by an induction coil distance 10 cm.
b. same as in a, but 30 min. later.

R.P. = intrapulmonary pressure.
V.O. = venous outflow from the left auricle.

TABLE IV.

STIMULATIONS OF THE C.V.S.

No. of Guinea Pig	Pepf. Pressure	Length of Coil	Initial I.P.P.	Latent Period	Effect on I.P.P.	Initial Outflow	Latent Period	Effect on Outflow.
11	3 cm	(3) 0 cm	28 cm	Within 30"	+ ?	10 drops/30"	1 m.	-
		(6) 0 cm after 250 γ Eserive	29	"		14 "	1.25 m	-
.2*	5	(6) LCVS 0 cm 30"	15.5	"	+	6	-	0
		(8) LCVS 0 cm 30"	7.0	1.25	late +	7	1.25	late +
13	4	(2) 12 cm.	19 .25	Within 30"	-	11	-	0
		(3) 12 cm.	16 .75	3.5 m.	s - ?			
		(4) 12 cm.	16	Within 30"	+	11	Within 30"	0
14	3.5	(1) 10 cm.	21	" 30"	+	11	"	-
		(3) 10cm.1 min.	24.5	" 30"	+	10	45"	-
		(5) 10 cm.	25	30"	+	10	45"	s -
15	3	(1) 10 cm.	19.5	30"	+	9.5	Within 30"	-
		(2) 15.5 cm.	"	30"	+	9.5	" 30"	-
17	6	(2 & 3) 0 cm.	17	30"	+	11	30"	-
21	4	(1) 10 cm.			+			0
		after Ergotoxine with no previous response						
19	5	(13) 4 cm.		1 min.	-			
		(14) 4 cm.		3 min.	-			

C.V.S. Cervical vago-sympathetic Nerves.
* Only the left cervical vago-sympathetic Nerve (LSVS) was stimulated.

IV. Nerve Stimulations. (Cervical vaso-sympathetic nerves.)

As stated earlier the insensitivity of the preparations and the early onset of broncho constriction was a serious obstacle to nerve stimulation experiments. This was particularly the case where the cervical vago-sympathetic nerves (C.V.S.) were separated into the cervical vagi (C.V.) and cervical sympathetic (C.S.) components; and these consequently have not yielded any constant results. Altogether 29 stimulations of the C.V.S. were made and 16 responses obtained. The results of these stimulations is given in Table IV and the following is a brief analysis of the main effects.

I.P.P. +	V.O. -	in 7 observations	(Figs.15,16,17,19).
I.P.P. +	V.O. 0	3	Fig. 18.
I.P.P. -	V.O. 0	1	Fig. 19.
I.P.P. +	V.O. +	1	Fig. 18.

It will be seen that although the more usual vagal effect of C.V.S. stimulation is a rise in I.P.P. associated with a fall in V.O. on occasions the change in outflow may not occur, moreover in one experiment a very definite fall in I.P.P. took place without any change in outflow (Fig . 19). In this experiment a subsequent repetition of the stimulus (with the coil distance unaltered) produced the more common effect of I.P.P. + and V.O. - . One of two interpretations may be/

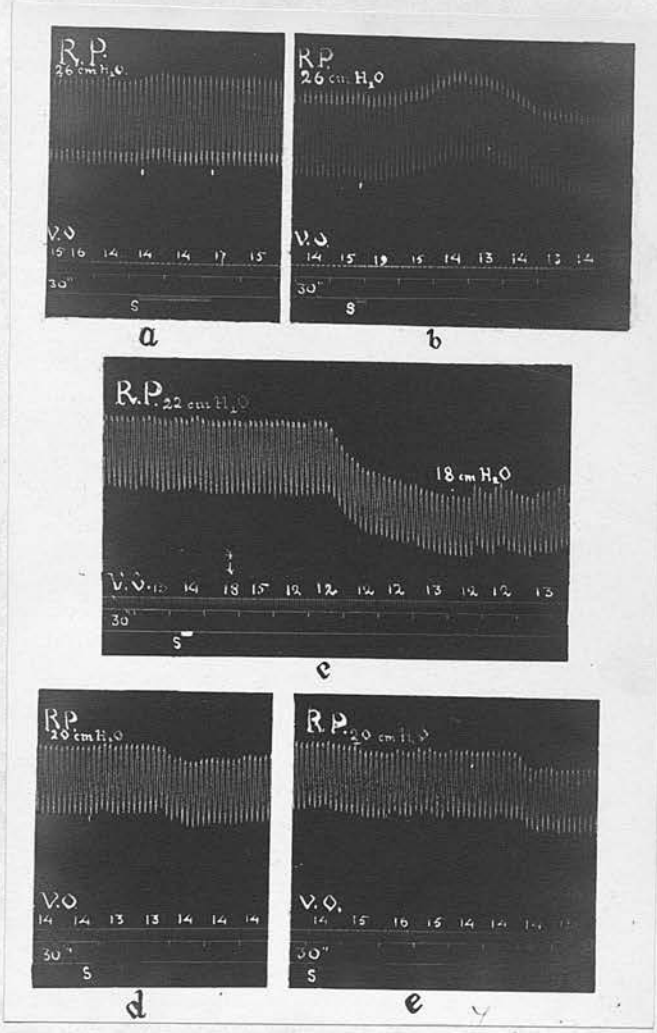


Fig. 20.

FIG. 20. 6.5.37. Guinea-pig 19. To show the effect of Ergotoxine on the effects of Adrenaline and nerve stimulations on the intrapulmonary pressure in the Isolated Pertused Lungs of a guinea-pig.

a. stimulation of the peripheral end of cut right cervical vago-sympathetic; coil distance = 0 cm.

b. At signal 5 γ adrenaline hydrochloride injected into the pulmonary arterial tubing.
Between b. and c. 300 γ Ergotoxine Ethanesulphonate were injected into the venous reservoir, and 15 min. were allowed to pass before c.

c. At signal 5 γ of Adrenaline hydrochloride were injected into the pulmonary arterial tubing.

d. At signal the peripheral end of cut left cervical vago-sympathetic was stimulated with coil distance 4 cm.

e. The same as d. 5 min. later.

be placed upon this result. Either the responsiveness of the bronchial muscle had changed during the experiment, or the sensitivity of the broncho dilator (probably sympathetic) fibres in the C.V.S. had declined and allowed full play to the effects of stimulation of the vagal broncho constrictor fibres. We are unable, because of insufficient evidence, to decide between the two interpretations. A third probability which may be examined depends upon the I.P.P. change being due to a spontaneous variation, but we believe that the extremely abrupt onset of change in I.P.P. militates against this hypothesis.

Whatever the nature of the mechanisms involved in the production of the I.P.P. change it seems clear that the V.O. is not always dependent upon the I.P.P. change because on rare occasions the two effects are dissociated.

We next proceeded to separate the vagal and sympathetic portions of the cervical vagus and to stimulate them separately. Before proceeding to discuss these results we wish to report on the effect of ergotoxine on changing the response to C.V.S. stimulation.

Fig. 20 shows that excitation of the C.V.S. on the injection of adrenaline causes a slight rise in I.P.P. and a certain change in the V.O. The rise to 38 and 36 drops / min. in V.O. in tracing b. and c. respectively/

TABLE V.

STIMULATIONS OF THE CERVICAL VAGI (C.V.).

Guinea Pig Number.	Stimulation and length coil.	Initial I.P.P.	Effect on I.P.P.	Effect on the venous outflow.
17	(2) Left C.V. 0 cm.	17 cm.	+	-
	(3) " " " 0 cm.	21.5	+	-
20	(6) Right C.V.12 cm→8 cm.	18.5	+	0 ?
22	(1) Both C.V. 6 cm.	23	+	-
	(4) " " 0 cm.	25	+	-
	400 γ Ergotoxine.			
	(9) Both C.V. 3 cm.→0 cm.	25	-	0 then Sl +

STIMULATIONS OF THE CERVICAL SYMPATHETICS (C.S.)

21	(3) Both C.S. 5 cm. Ergotoxine 400 γ	-	+	?
	(5) " " 5 cm.		0	0
	(6) " " 0 cm.		-	0
19.	(17) Left C.S. 0 cm.	28	-)*	0
	(18 & 19) Left C.S. 0 cm.	28	-)	0 or - ?

* After 300 γ Ergotoxine ethanesulphonate.

respectively is due to the amount of fluid injected into the pulmonary arterial tubing. Subsequent to the addition of ergotoxinethanesulphonate (300γ) into the venous reservoir, both adrenaline and C.V.S. stimulation reduced the I.P.P. and had little or no effect on the V.O.

When we consider the previously described effect of adrenaline in causing a rise in I.P.P., one which appears peculiar to the closed-circuit perfused guinea-pig lungs, we are inclined to interpret the results in Fig. 20 as being in part due to the stimulation of adrenergic fibres running in the C.V.S. nerves, which under the conditions of the experiment cause a broncho-constrictor response. What is more important perhaps from the point of view of our thesis is that in this particular experiment we have observed both a rise and a fall in I.P.P. due to stimulation of the C.V.S.,yet there is no significant change in the venous outflow; therefore broncho motor effects can take place without a passive influence on the vascular system.

Table V summarises the effects of separate stimulations of the cervical vagus and the cervical sympathetic nerves. The cervical vagus excitations produced a rise in I.P.P. and a fall or no change in the venous outflow (Fig. 21); whereas in one experiment cervical sympathetic fibre stimulation gave rise to an augmented V.O. with an uncertain effect on the I.P.P. (Fig.25)

One/

FIG. 16. 25.3.37. I.P.L. of guinea-pig 15. At arrow the peripheral ends of the cut cervical vago-sympathetic nerves on both sides were stimulated with coil d = 10 cm. The effect is a rise in I.P.P. and a fall in V.O. This response is similar to the ACH response.

FIG. 17. 25.3.37. I.P.L. of guinea-pig 16. At arrow the peripheral ends of the cut right and left cervical vago-sympathetic nerves were stimulated with coil d = 8 cm. The typical I PP + VO - result is again produced as in Fig. 16.

FIG. 18. I.P.L. of guinea-pig 12. At (1) 0.01 ACH. causes a rise in 1.P.P. and a fall in V.O. Six minutes later at (2) the peripheral ends of cut right and left cervical vago-sympathetic nerves were stimulated with coil distance 0 cm. The result is a rise in 1.P.P. and no change in V.O. At (3) the nerve stimulation was repeated as in (2) and a small rise in I.P.P. was associated with a sharp increase in V.O. lasting 1 minute. This sudden V.O. release may have been due to mechanical causes.

FIG. 19. 19.3.37. I.P.L. of guinea-pig 13. At (1) and (2) the peripheral ends of the art. right and left cervical vago-sympathetic nerves were stimulated with coil d 12. At (2) the typical ACH response is obtained (1.P.P. + VO -), but at (1) the I.P.P. falls whilst the V.O. does not change. This is not only a further example of the "dissociation" of 1.P.P. and V.O. effects but it also shows that in the same experiment two dissimilar results may be obtained in response to similar stimulations.

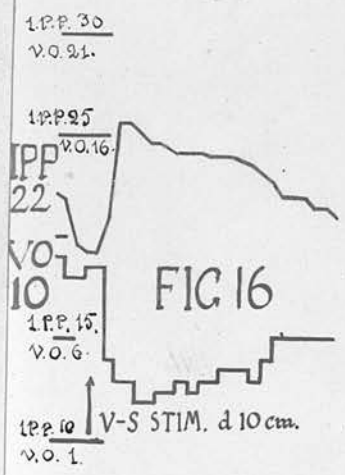


FIG 16

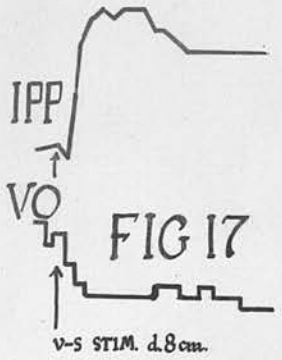


FIG 17

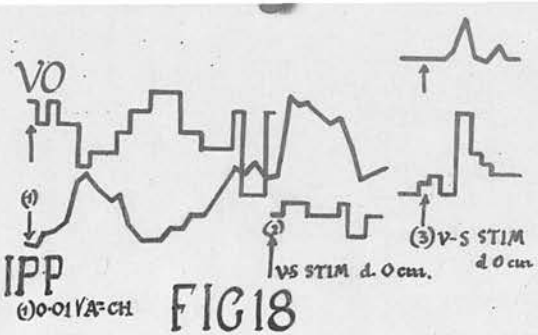


FIG 18

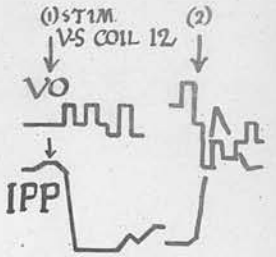


FIG 19

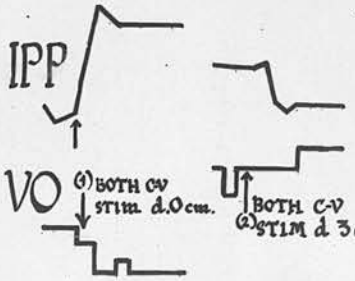
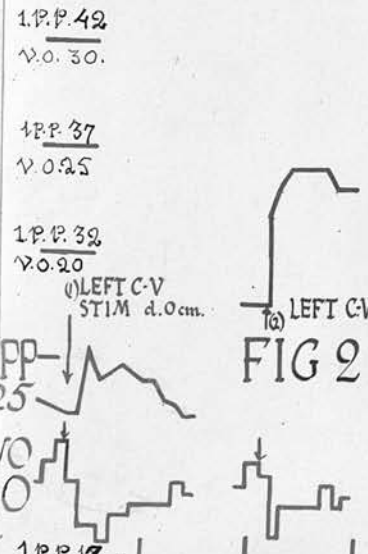


FIG 22



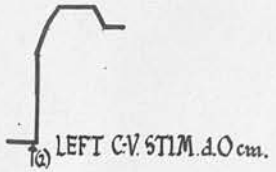
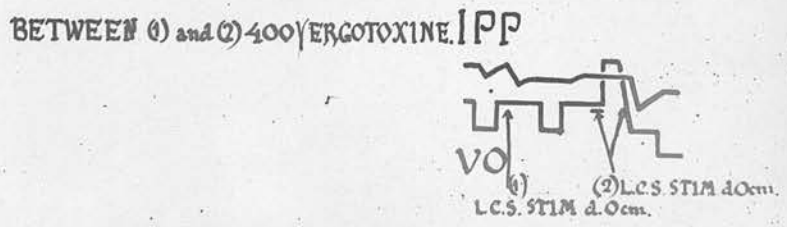


FIG 21

FIG 25



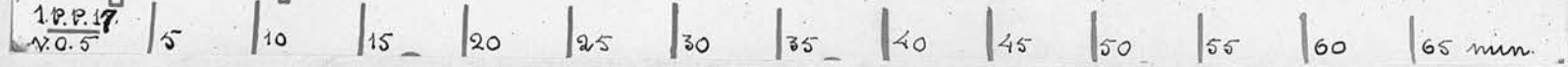


FIG. 21. 27.4.37. I.P.L. of guinea pig 17. At (1) and (2) the peripheral end of cut left cervical vagus was stimulated with coil d = 0 c.m. In response to the first stimulation I.P.P. rose and V.O. fell, and in response to the second a rise in 1.P.P. took place without any change in V.O.

FIG. 22. 21.5.37. I.P.L. of guinea-pig 22. At (1) peripheral ends of cut right and left cervical vagi were stimulated with coil distance 0 cm. A rise in I.P.P. with an associated fall in V.O. resulted. Between (1) and (2) 400 γ Ergotoxine were injected into the venous reservoir and 10 min. was allowed to pass; at (2) the peripheral ends of cut right and left cervical vagi were again stimulated with coil d 3 cm. 0 cm. No change in V.O. was produced but the original 1.P.P. rise was converted to an 1.P.P. fall.

FIG. 25. 6.5.37. I.P.L. of guinea-pig 19. (1) and (2) are two successive stimulations of peripheral ends of cut right and left cervical sympathetic nerves. The sympathetic components had been separated from the vagal in the cervical vago-sympathetic nerves. Coil d = 0 cm. No clearly defined changes are produced. A slight fall in I.P.P. follows each stimulation. A short spate in venous outflow takes place in response to (a).

I.P.L. = isolated pertused lungs. P.A.= pulmonary artery.
I.P.P. = intrapulmonary pressure. V.O.= venous outflow.
ACH. = acetyl choline. Adr. = adrenaline.

FIG. 23. 21.5.37. Guinea-pig 22. Isolated Pertused Lungs Preparation.

a. at signal S, both cervical vagi stimulated; coil d = 0 cm. between a. and b. 400 γ Ergotoxine Ethanesulphonate injected into the venous reservoir. 35 min. allowed to pass before stimulations in b.

b. at signal S_1 , both cervical vagi stimulated; coil distance 3 cm.
at distance S_2 , " " " " "
distance 0 cm.

Signal

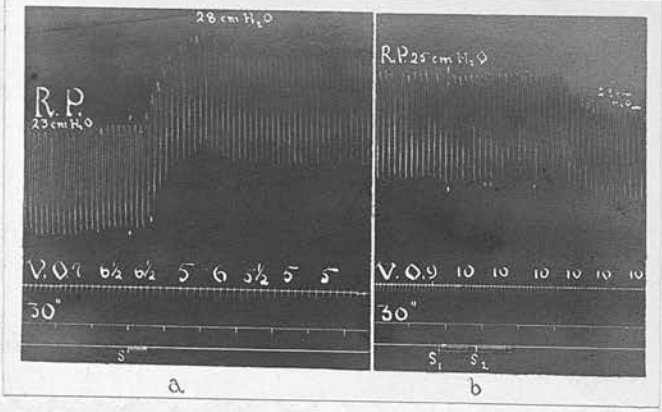


Fig. 23.

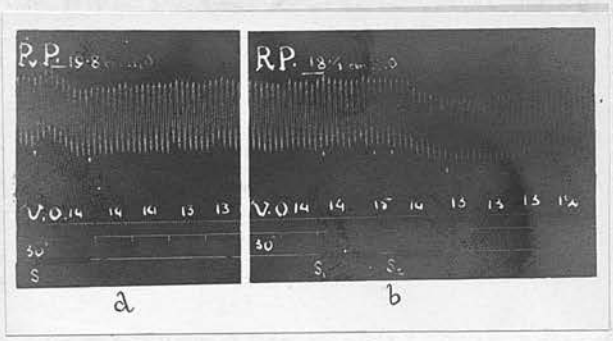


Fig 24.

FIG. 24. 6.5.37. Guinea-pig 19. Isolated Pertused Lungs.

a. at S, stimulation of the peripheral end of cut left cervical sympathetic, coil distance 12 cm.

b. at S_1 and S_2, two successive stimulations of the peripheral end of cut left cervical sympathetic, coil distance 0 cm. in both cases.

Prior to a. and b. stimulations of left cervical sympathetic had no effect 20 min. before a. and b. 300 γ Ergotoxine Ethanesulphonate was injected into venous reservoir.

One remarkable fact emerges from these experiments, namely, stimulation of the C.V. or the C.S. nerves cause a broncho constriction but after ergotoxine a broncho dilatation. (Figs. 22, 23, 24).

In the light of these experiments the number of interpretations which can be placed upon the mechanisms responsible for broncho constriction assumes somewhat large proportions, and it would be idle to speculate at length in the absence of more complete evidence than that which we have presented. We shall therefore content ourselves with only a brief survey.

As a starting point one must assume that for some reason, as yet unknown, adrenaline, in some of the experiments, causes broncho constriction. If, therefore, there are adrenergic fibres in the C.V.S. and C.S. then their stimulation would be expected to produce broncho-constriction under the conditions imposed upon the lungs by the closed-circuit perfusion technique. If this assumption is correct then stimulation either of the C.V. or of the C.S. or of the C.V.S. as a whole, will give rise to broncho constriction, in part due to the stimulation of cholingeric fibres giving the normal broncho constriction response, and in part to excitation of the adrenergic fibres, giving an abnormal broncho-constriction response. Now the latter effect may quite well be reversed by ergotoxine so that adrenergic fibre stimulation in ergotoxinised preparations would cause broncho dilatation/

broncho dilatation. There is now a difficulty to be overcome, because stimulation of the C.V. nerves which are supposed to contain only cholinergic broncho constriction fibres, produce broncho dilatation in ergotoxinised preparations. It seems, therefore, that we are drawing towards one of two conclusions; that either ergotoxine reverses the broncho constriction of cholinergic fibres, or there are adrenergic fibres in the C.V.,stimulation of which gives a broncho constriction in closed-circuit perfusion preparations, and after ergotoxine a broncho dilatation.

Since we have insufficient data to correlate the abnormal adrenaline broncho constriction response with the effect of nerve stimulations, and have no definite evidence as to the correctness of our assumptions regarding the nature of the broncho constrictor and dilator nerves in the guinea-pig, the answers to the questions raised can only be given by further experiments.

It is difficult to decide whether the rise of I.P.P. in these observations is due to constriction of plain muscle in the air passages situated in the territory common to the pulmonary and bronchial circulations, or whether the contraction affects the actual air sacs or the interstitial plain muscle. Each or a combination of these might produce a rise in I.P.P. with an associated/

associated decrease in venous outflow resulting from purely mechanical causes. To what extent the respiratory pressure and venous outflow are independent cannot yet be estimated, but that they do occur so cannot be disputed.

In most observations with A.Ch. injections and stimulations of the C.V.S. nerves, a diminution of V.O. occurs simultaneously with a rise in I.P.P. This by itself suggests that the reduction of flow is a purely passive effect of the broncho constriction on the blood vessels. But in the adrenaline experiments we are provided with instances where no change in I.P.P. is accompanied with a diminution of outflow (3 cases) where an I.P.P. change is accompanied by unassociated or uncertain flow changes (3 cases) and in one experiment both the I.P.P. and V.O. varied in the same direction. Therefore although flow changes do occur in a manner which suggests they are dependent upon mechanical factors, they also take place in a number of observations in a manner absolutely dissociated from I.P.P. pressure responses. This suggestion that flow mechanisms and those of the intrapulmonary pressure, although possibly mechanically interrelated, are inherently independent, is further supported by an examination of spontaneous changes which frequently occur in guinea-pig preparations. These show that flow changes frequently/

frequently do take place together with I.P.P. variations - giving the inverse ratio "effect" i.e. I.P.P.+-, V.O. -+ ; but also it frequently happens that the I.P.P. variations take place whilst the flow is undisturbed; and in one instance I.P.P. fall occurred with an associated V.O. diminution. Flow changes independent of intrapulmonary pressure variations occur only very seldom.

It is concluded therefore that I.P.P. and V.O. variations take place in a manner explainable on "the purely mechanical" interdependence theory, but that they can be separately altered, spontaneously, or by nerve stimulation, or by drug injections.

A support to this view is given by previous investigators.- Dale and Narayana (1935) come to the conclusion that in their responses to Acetyl Choline and Adrenaline injections the vasomotor and the broncho- o/ motor mechanisms are mutually independent. Daly, Peat and Schild (1935) probed the same question while investigating responses of guinea-pig lung to histamine. They concluded that although "throughout their investigations they have generally encountered a vascular constriction associated with the broncho constriction of anaphylactic shock" still they (1) observed slight vascular responses which occurred without any detectable alterations in I.P.P. and (2) that the broncho constriction of shock may be well sustained whilst the vascular constriction/

constriction shows good recovery.

Further corroborative evidence is obtained from experiments on other animals. Working on dogs Daly and Euler (1932) stated that excitation of certain vasomotor nerves to the lungs may give rise to bronchoconstriction associated with pulmonary vaso-dilatation, ormvaso-constriction; thus demonstrating some independence of the two mechanisms. R.P. Alcock (Ph.D. Thesis Edinburgh 1936); showed that in the rat the same dissociation of vascular and respiratory pressure responses sometimes took place in experiments with Adrenaline, Acetyl Choline or Histamine.

SUMMARY.

In isolated pertused lungs of the guinea-pig

(1) Acetyl Choline raises the respiratory pressure and diminishes the venous outflow; both effects are abolished by Atropine.

(2) In general stimulation of the peripheral and of the cervical vago-sympathetic nerves causes a similar rise of intrapulmonary pressure and a diminution in outflow. In exceptional cases the I.P.P. falls, an effect which may be due to concomitant stimulation of the cervical sympathetic nerves.

(3) Adrenaline leads to a variety of responses: a rise of I.P.P. accompanied by a fall in V.A. being the most frequently found. Five out of the total of 14 responses were in favour of the independence of the vaso- and bronchomotor mechanisms. In one instance an I.P.P. rise occurred together with an V.O. increase.

(4) Stimulation of the peripheral end of the cut C.V. usually causes a rise in I.P.P. associated with a diminution in outflow.

(5) Only 3 responses were obtained with stimulations of the peripheral ends of cut cervical sympathetics. In one a rise in I.P.P. was obtained; the flow was not recorded. In other two responses occurred after/

after an administration of Ergotoxine - a fall in I.P.P. with no change or a questionable diminution of outflow are recorded.

(6) Ergotoxine reverses the effects of stimulation of the cervical vagus converting an I.P.P. + V.O. - to an I.P.P. - R.P.sl.+; as also the respiratory pressure rise to an I.P.P. fall when the entire C.V.S. is stimulated.

(7) Examination of spontaneous variations shows that V.O. and I.P.P. changes may occur in an unassociated manner; and from this and all data from above observations it is concluded that the vasomotor and respiratory pressure mechanisms are often mechanically affecting each other, and are in fact entirely independent.

SECTION 2.

NERVE STIMULATIONS OF CONTROLLED FREQUENCIES.

EXPERIMENTS AND DISCUSSION.

In this series of seven experiments an attempt was made to stimulate the cervical vagal and sympathetic nerves to the lungs with a view to obtaining a correlation between any specific range of frequencies, the strength of stimulus remaining approximately constant, and types of I.P.P. and V.O. response. These experiments followed immediately upon those of section one, therefore with the exception of the introduction of the new frequency stimulation apparatus, and in 5 experiments of anaesthesia, the experimental conditions remained the same.

Anaesthesia was used in five experiments, and with so few results on record it would be impossible to say what additional influence the type of anaesthesia had on the type of response. As will be described later it was found that the anaesthetising of guinea-pigs is a very lengthy process, and it would be very improbable if no interference on bronchial and vascular responses proceeded from that source. Out of the five experiments we did perform the more sensitive preparation was obtained with ether.

An ordinary Du Bois Raymond coil (Palmer & Co. Ltd.) gives a variable number of stimuli depending on the/

TABLE I.

No. of Guinea-Pig	Date	Anaesthetic Used.	Nerve Structure Stimulated.	Frequency.	Coil Distance	Duration of Stimulus	Serial of Observation.	Change in Venous Outflow	Change in I.P.P.
23	24.6.37	-	C.V.	20	9	90"	1	-	+ +
				40	9	108"	2	0	+
				400 γ ESERINE.					
				40	G	108"	3	Sl -	+ prolonged - or ?- Sl +
				20	9	90"	4	0	0
				20	9	78"	5	0	0
				40	9	120"	6	0	0
24	24.6.37	-	C.V.S.	9	10	60"	2	-	+
			C.V.S.	40	10	72"	3	0	- ?
			C.V.S.	40	10	66"	4	0	+
				400 γ ERGOTOXINE.					
			C.V.S.	40	10	72"	5	0	0
			C.V.S.	9	10	72"	6	Sl +	0
30	1.7.37	Urethane 2.3 gm/kg	C.V.S.	8.7	10	30"	1	0	0
			C.V.S.	18	10	30"	2	0	0
				39	7	30"	3	0	0
				39	11	30"	4	0	0
			C.S.	39	9	30"	5	0	0
			C.S.	87	9	30"	6	0	0
			C.S.	87	9	30"	7	0	0

TABLE I.

No. of Guinea-Pig	Date	Anaesthetic Used.	Nerve Structure Stimulated.	Frequency.	Coil Distance	Duration of Stimulus	Serial of Obser-vation.	Change in Venous Outflow.	Change in I.P.P.
31	5.7.37	Urethane 1.57gm/Kg.	C.V.S.	817	9	66"	1	0	0
			C.V.S.	87	9	78"	2	0	0
			C.V.S.	8.7	9	72"	3	0	+ Sl.
			C.V.S.	8.7	9	66"	4	0	Sl. +
			C.V.S.	8.7	9	66"	5	0	0
			C,V.S.	87	9	66"	6	0	? + Sl.
			C.V.S.	8.7	9	66"	7	0	? + Sl.
33.	15.7.37	Chloral Hydrate 1gm/Kg.	C.V.S.	No response to d		7 and 9	∫ 8.7, 87, 70 ; 7 stimulations		
34.	15.7.37	Ether	C.V.S.	87	9	90	2	0	+
			C.V.S.	70.8	9	45	3	0	+
			C.V.S.	51.5	9	45	4	-	+++
			C.V.S.	38.8	9	45	5	-	++
			C.V.S.	19.4	9	60	6	?+ -	? -+
			C.V.S.	87	9	60	7	0	?+
			C.V.S.	19.4	9	90	8	0	0
			C.V.S.	38.8	9	60	9	0	0
			Thereafter no effect even after Eserive.						
35.	7.7.37	Ether	Both C.V.S.	87	9	60"	2	0	+
			C.V.S.	51.5	9	60"	3	0	+ Sl.
			C.V.S.	19.5	9	66"	4	0	+ Sl.
			C.V.S.	87	9	72"	5	0	0
			Thereafter no effect.						

the coil distance. For break stimuli only the range is 40 - 80 /sec.; for make and break stimuli the range is 80 - 160 / sec. Now, such a coil has been used in all former stimulation experiments and also in the experiment in this section from which Fig.1 was taken. For controlled frequency stimulations we connected the coil to a 4 volt battery and a Palmer & Co. Rotary commutator which gives 46 break shock / rev. or 92 make and break shocks per rev. when both terminals are down. The 1 H.P. Motor supplied by 230 volts from the main rotated the commutator 34 times / min. By an arrangement of pulleys from the motor to the commutator and by using one or both terminals the following frequencies per second could be conveniently used: 8.7, 19.38, 38.3, 51.5, 70.8, 87. The coil distance range for break shocks was 13-7 cm.

Altogether 35 stimulations of the C.V.S. were made. Of the 18 negative and the 17 positive responses present on analysis the following picture was made:-

I.P.P.	V.O.	No. of Tests.	Frequencies/sec. used.
+	-	3	9, 38.8, 51.5
+	0	8	40,8.7, 19, 51, 70, 87.
+(-?)	-(+)?	1	19.4
+ ?	0	3	8.7, 87
- ?	0	1	40
0	sl +	1	9

It/

It will be seen from this analysis and from the detailed table I, that it is impossible at the present stage with the few experiments performed to correlate the nature of a bronchial and vascular response with any specific frequency of stimulation, nor to say which frequency is as a rule optimum for the dissociation of the two lung mechanisms. However in experiment 34, (Figs. 3 and 4) it is suggestive that a dissociation of these responses might have been produced at high frequencies viz. 87/sec., and 70.8/sec., and also with the low frequency viz. 19/sec.; whereas a strong I.P.P. + and V.O. - response resulted at frequencies 51.5/sec. and 38.8/sec. It is possible that in this experiment the diminution of flow in test (5) is still a continuation of its fall produced by stimulation at (4), but we incline to the view that the V.O. fall in (5) is an independent response.

It would seem that the type of response was not affected by the different coil lengths within the narrow range used, 9-11 cm.; but it would appear that the period of stimulation in order to be effective should exceed 30 sec. After that the response, if any, commences instantaneously.

When these experiments are taken as a group the important and obvious fact which emerges is that the results produced agree well with those obtained on

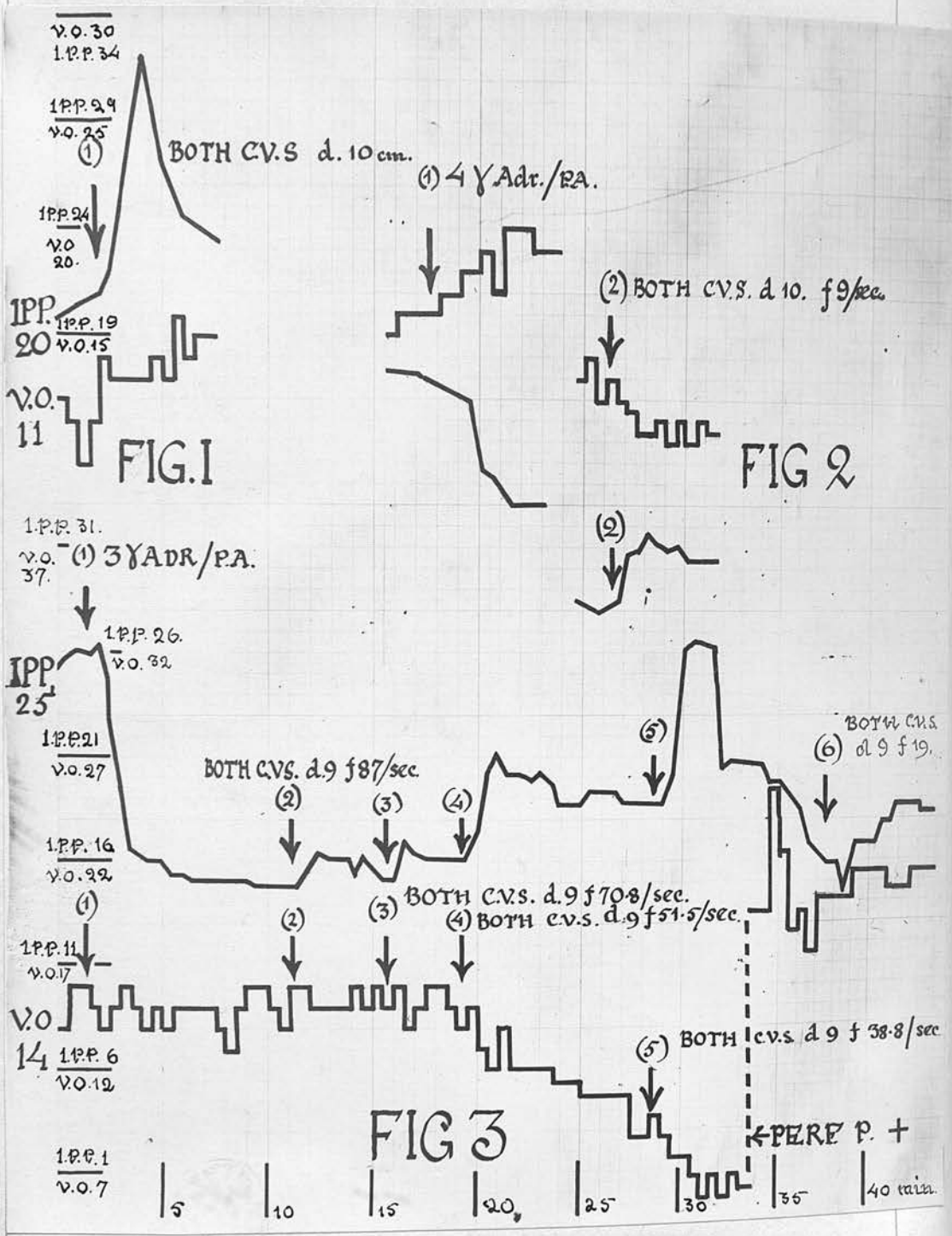

V.O. 30
I.P.P. 34
I.P.P. 29
V.O. 25
(1)
BOTH C.V.S d. 10 cm.
I.P.P. 24
V.O 20.
IPP 20
I.P.P. 19
V.O. 15
V.O. 11
FIG. 1
(1) 4 γ Adr./P.A.
(2) BOTH C.V.S. d 10. f 9/sec.
FIG 2
I.P.P. 31.
V.O. 37.
(1) 3 γ ADR/P.A.
(2)
I.P.P. 26.
V.O. 32
IPP 25
I.P.P. 21
V.O. 27
BOTH C.V.S. d. 9 f 87/sec.
(2)
(3)
(4)
(5)
BOTH C.V.S.
(6) d 9 f 19.
I.P.P. 16
V.O. 22
(1)
I.P.P. 11
V.O. 17
(2)
(3) BOTH C.V.S. d. 9 f 70·8/sec.
(4) BOTH C.V.S. d. 9 f 51·5/sec.
V.O 14
I.P.P. 6
V.O. 12
(5) BOTH C.V.S. d 9 f 38·8/sec.
FIG 3
←PERF P. +
I.P.P. 1
V.O. 7
5
10
15
20
25
30
35
40 min.

FIG.1. 20.6.37. Guinea-pig 22. At (1) the peripheral ends of both cut C.V.S. were stimulated with coil d. = 10 cm. The I.P.P. rose and the V.O. also slightly increased.

FIG. 2. 24.6.37. Guinea-pig 24. At (1) 4 γ Adrenaline injected into the P.A. produced a fall in I.P.P. associated with an increase in venous outflow.
At (2) the peripheral ends of both cut C.V.S. were stimulated with coil d. 10 f 9/sec. An increase of I.P.P. took place and was associated with a fall in the V.O.

FIGS. 3 and 4. 15.7.37. Guinea-pig 34. Etherised.
At (1) 3 Adr. injected into the P.A. produced a fall in I.P.P. unaccompanied by any change in V.O.
At (2) Both C.V.S. were stimulated with coil d 9 f 87.
At (3) Both C.V.S. were stimulated with coil d 9 f 70.8
In (2) and (3) a small rise in I.P.P. took place without an accompanying change in V.O.
At (4) a stimulation of both C.V.S. with coil d 9 f 51.5 caused a big rise in I.P.P. and a large decrease in V.O.
At (5) a stimulation of both C.V.S. with coil d. 9 f 38.8 again caused a big rise in I.P.P. and a further decrease in V.O.
At (6) a stimulation of both C.V.S. with coil d. 9 f 19 produced a rise in I.P.P. without any change in V.O.
Between (5) and (6) the Perfusion pressure was raised through 3 cm. H_2O.

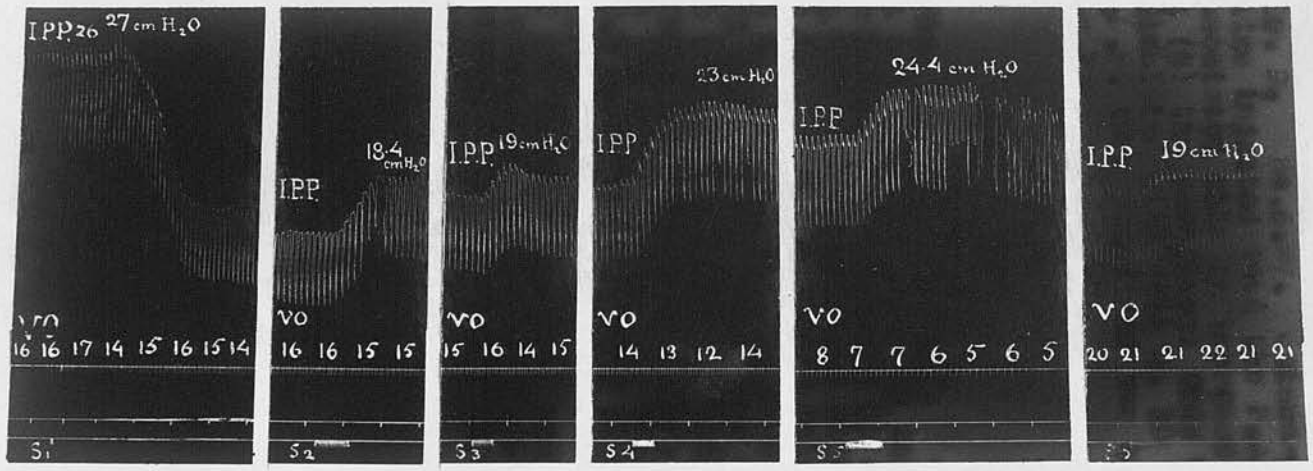

I.P.P. 27 cm H2O
VO
16 16 17 14 15 16 15 14
S1
I.P.P. 18.4 cm H2O
VO
16 16 15 15
S2
I.P.P. 19 cm H2O
VO
15 16 14 15
S3
I.P.P. 23 cm H2O
VO
14 13 12 14
S4
I.P.P. 24.4 cm H2O
VO
8 7 7 6 5 6 5
S5
I.P.P. 19 cm H2O
VO
20 21 21 22 21 21

Fig 4.

C.V.S. and C.V. stimulations in Section I of this thesis. It is obvious that in all clear cut tests a rise in I.P.P. is produced. This occurs in 12 out of 17 tests, and this rise may be accompanied by a fall in V.O. (Fig.2, test (2), fig. 3 and 4, tests (4) and (5)), or it may not be accompanied with any change in outflow. Fig. 3 and 4, tests (2) (3) and (6). This latter I.P.P.+ V.O.+ response in figure 3 takes place both at low and at raised perfusion pressures. These tests present clearly defined instances in favour of the inherent independence of the bronchial and vascular lung mechanisms; but to what extent the vascular responses, which are particularly rare in this set of experiments, have been depressed by the use of anaesthesia, it is impossible to estimate. In 3 other tests an uncertain rise in I.P.P. took place without any change in outflow. A questionable fall in I.P.P. with no change in V.O. was observed once; and no change in I.P.P. with a concomitant slight increase in V.O. was seen also once. The former can be composed to the anomalous response in Fig.19, where C.V.S. was stimulated, and the latter to the one in fig.25, test (2) where L.C.S. was stimulated with strong current. In discussing Fig.19 and Fig.25 it was suggested that these reactions were due to sympathetic adrenergic fibres, and it is significant that both these last reactions occurred in the same preparation.

The/

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The three stimulations of separated cervical sympathetics yielded negative results; but stimulations of the C.V. in one non-anaesthetised preparation brought fruitful results. Altogether 6 tests were made with only 2 negative results.

C.V. stimulations.

I.P.P.	V.O.	No. of Tests.
++	-	1
+	sl -	1
+	0	1
-? sl +	0	1
0	0	2

It will be seen that clearly the predominant response is a rise in I.P.P. with or without an associated fall in V.O. This again is in full agreement with the C.V. stimulations of earlier experiments.

In this section in Fig.1 is shown another example of a very rare type of dissociation of bronchial and vascular responses where a big rise in I.P.P. was accompanied by a small change in V.O. in the same direction. It has been observed once in spontaneous variations, which are so typical of a closed circuit perfusion preparation, and in Fig.18 where it was produced by a stimulation of the C.V.S.

In Figs. 2 and 3 two responses to Adrenaline are recorded. The former shows the more usual I.P.P.- V.O.+ type of response which has been so often observed by/

by ourselves and by Dale and Narayana (1935). In Fig. 2 the Adrenaline injection produced an I.P.P. fall without any change in V.O., in a manner similar to that shown in Figs. 13 and 14. This has been earlier explained on the assumption that Adrenaline produced a relaxation of the bronchial muscle but that the vascular territory affected by adrenaline was already in a fully dilated state, and therefore could not be passively further dilated. This again brings us back to the conclusions formerly made by Dale and Narayana.

All these above results further bear out the main theme of this thesis. That the bronchial and vascular mechanisms may be inherently independent but that in a great many circumstances they mechanically modify each other's behaviour.

SUMMARY.

In the perfused lungs of a guinea-pig, using the recirculation method:

(1) Types of responses on stimulation of C.V.S., C.V., and C.S. in the neck cannot be correlated to any specific frequencies of stimulus.

(2)Urethane, Chloral Hydrate and Ether have been used in five experiments to produce general anaesthesia. It is suggestive that their use was associated with a lessened sensitivity of the preparation, particularly of the vascular responses.

(3) Three stimulations of the C.S. yielded negative results.

(4) Stimulations of the C.V.S. and C.V. yielded results which agreed well with those obtained on similar stimulations in the earlier part of the thesis. Predominantly their stimulation leads to a rise in I.P.P. with or without an associated fall in V.O.

(5) The stimulation tests and the two results produced by injections of Adrenaline clearly point out that a dissociation between the bronchial and vascular responses in the lungs of a guines-pig can be repeatedly demonstrated in a variety of circumstances.

APPENDIX:

A NOTE ON THE USE OF ANAESTHETICS IN GUINEA-PIG PREPARATIONS.

A NOTE ON THE USE OF ANAESTHETICS IN GUINEA-PIG PREPARATIONS.

In the course of our experiments some information was obtained in the use of anaesthetics in guinea-pig lung preparations. It was thought that if the dissection of nerves were performed under an anaesthetic, and then the animal allowed to remain under it for some time, then the circulating blood would have time to remove any of the bronchoconstrictor substances responsible for the rigidity in the lungs, and that by this means a sensitive preparation would be obtained.

Anaesthesia in perfusion preparations is always a danger since it is uncertain which vital link in the mechanism of the reactions would be influenced by it. As stated in the last section the insensitivity of the preparations, and particularly the loss of reactivity on the part of the vascular bed has been in part at least attributed to the influence of the anaesthetics used.

We could find little information regarding the methods of anaesthetising guinea-pigs, but in all we performed 9 such experiments. The animals were all famale of the same strain as used in the earlier experiments, and the procedure of dissection, stimulation, and perfusion also exactly as described in earlier sections.

Pernocton/

Pernocton (447 F) was used in two experiments. In the first one (30.6.37) a total of 1.82 cc./Kg Wt. was given in 4 multiple injections in the course of 5½ hrs. The animal became limp within the first 30 min. but was too sensitive to allow dissection. It died of its own accord 5½ hrs. after the first injection. The second guinea-pig (29.6.37) had a total of 1.15 cc./Kg Wt. given to it in multiple injections, but although it became limp and dazed within the first 10 min., 2 hrs. 20 mins. after the injection it was still too sensitive, and had to be killed by a blow on the neck.

Pernocton was not used again, and the next experiment was performed with Nembutal. (30.6.37) A solution of powder was made in distilled water in concentration of 1 grain/cc. A total of 1 grain/Kg Wt. was injected intraperitoneally in 3 portions. The animal developed a continuous tremor of limbs, and extreme sensitiveness to pricking and tapping. Respirations quickened to 65-70/min., and the heart rate was on the average 200/min. Eye reflexes were present throughout. At the end of 2½ hrs. the animal was killed by a blow on the neck, and the cervical nerves dissected. Extreme rigidity of lungs appeared, and the experiment was a negative one as no responses in I.P.P. could be obtained and the V.O.was negligible.

Urethane/

Urethane was used twice: it was injected intraperitoneally. A total of 2.3 grams/Kg Wt., and 1.57 grams/Kg Wt. on 1.7.37 and 5.7.37 respectively. These experiments were recorded in the last table. The methane seems to affect the animals rapidly as far as general limpness could show, but breathing became very irregular and continuous tremor of limbs was apparent in both cases. The pupils were widely dilated. After 3 hrs. 45 min. in the first experiment the animal was killed by dissection, but 10 Adrenaline was injected into the heart shortly before death. The lungs were very elastic, but on stimulation no responses were obtained. The second Urethane preparation yielded two slight rises in I.P.P., and two questionable ones. The venous outflow showed no responses to any stimulations made. This animal showed the same reactions during anaesthesia as the first one, but it remained only 2 hrs. before being ready for dissection, and no adrenaline was injected during the making of the preparation.

Two attempts to use Chloral Hydrate (50% solution) were made. The first animal received an injection of 1 gram/Kg Wt.; the anaesthetic acted rapidly and an hour later the nerves were dissected out and the preparation set up. The lungs were particularly elastic, but no response could be obtained to nerve stimulations either in I.P.P. or V.O.

The/

The second guinea-pig received 0.83 gram/Kg Wt. in two injections. In contradistinction to the first one, this animal became soon very sensitive and spasmodic. After 5 hours in this condition the guinea-pig was returned to the animal-house, where it recovered overnight, and on the following day showed quite a normal behaviour.

The best results were obtained with Ether in guinea-pigs 34 and 35. (Section 2, Table 1). Ether acts quickly, but during its administration the animal struggles violently. An interesting observation was made that when completely insensitive to skin stimuli the animal jerks spasmodically when lowered rapidly through 5 feet. With the best ether preparation the cervical vago-sympathetics were first dissected out and then the preparation was allowed to remain undisturbed for half an hour, after which the chest was opened and the dissection and preparation completed. The second guinea-pig died during the dissection of the left C.V.S. Both these preparations showed a relative sensitiveness of stimulation of the peripheral ends of cut C.V.S. The I.P.P. was particularly sensitive at the very beginning of the experiment.
In the first experiment four good responses were obtained, of I.P.P.+ ; two questionable increases; two definite reductions in C.O., and one questionable diphasic/

diphasic response in V.O. Thereafter no responses could be obtained even after Eserine. The second preparation gave 3 definite rises in I.P.P. but the V.O. showed no response whatever.

In conclusion one can say that with the variety of Anaesthetics we used, we could not obtain satisfactory results. The actual anaesthetising took too long a time, the animals remained too sensitive, or showed unusual reactions such as excessive tremors and spasms. In addition it is almost certain that anaesthesia affects the type of response to nerve stimulations, and most probably cuts out the responses of the blood vessels to a greater degree than those of the plain muscle of the bronchi.

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